In re Appln of Sogabe et al. Application No. 09/940,941

IN THE CLAIMS:

1.-23. (cancelled)

24. (currently amended) A creatine amidinohydrolase (i) encoded by a nucleic acid sequence obtained by mutation of (a) the nucleic acid sequence of SEQ ID NO:2 or (b) a nucleic acid sequence encoding the amino acid sequence of SEQ ID NO:1 and (ii) having the following physicochemical properties:

Action: catalyzing the following reaction:

creatine + $H_2O \rightarrow sarcosine + urea$

Heat stability: not more than about 50 °C (pH-7.5, 30 min)

Optimum temperature: about 40-50 °C (at a pH of about 7.5)

Km values for creatine in a coupling assay using a sarcosine oxidase and a peroxidase: 3.5-10.0 mM

Isoelectric point: about 4.5.

25. (currently amended) A creatine amidinohydrolase (i) encoded by a nucleic acid sequence obtained by mutation of (a) the nucleic acid sequence of SEQ ID NO:2 or (b) a nucleic acid sequence encoding the amino acid sequence of SEQ ID NO:1 and (ii) having the following physicochemical properties:

Action: catalyzing the following reaction:

creatine + $H_2O \rightarrow \text{sarcosine} + \text{urea}$

pH stability: being stable at pH 5-8 (40 °C, 18 h preservation)

Optimum pH: pH about 8.0-9.0 (at a temperature of about 37° C)

-Km-values for creatine in a coupling assay using a sarcosine oxidase and a

peroxidase: 3.5-10.0 mM

Isoelectric point: about 4.5.

- 26. (canceled)
- 27. (currently amended) The creatine amidinohydrolase of claim 24, which has the following physicochemical properties:

Optimum temperature: about 40-50 °C

Optimum pH: about 8.0-9.0 (at a temperature of about 37 °C).

28. (previously added) The creatine amidinohydrolase of claim 24, which has a molecular weight of about 43,000 (SDS-PAGE).

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- 29. (canceled)
- 30. (previously added) The creatine amidinohydrolase of claim 25, which has a molecular weight of about 43,000 (SDS-PAGE).
 - 31.-32. (canceled)
- 33. (currently amended) A creatine amidinohydrolase (i) encoded by a nucleic acid sequence obtained by mutation of (a) the nucleic acid sequence of SEQ ID NO:2 or (b) a nucleic acid sequence encoding the amino acid sequence of SEQ ID NO:1 and (ii) having the following physicochemical properties:

Action: catalyzing the following reaction:

creatine + H₂O → sarcosine + urea

Km values for creatine in a coupling assay using a sarcosine oxidase and a

peroxidase: 3.5-10.0 mM

Optimum temperature: about 40-50 °C (at a pH of about 6-8 7.5)

Optimum pH: pH about 8.0-9.0 (at a temperature of about 37° C)

Molecular weight: about 43,000 (SDS-PAGE)

Isoelectric point of 4.5.

- 34. (canceled)
- 35. (currently amended) A creatine amidinohydrolase (i) encoded by a nucleic acid sequence obtained by mutation of (a) the nucleic acid sequence of SEQ ID NO:2 or (b) a nucleic acid sequence encoding the amino acid sequence of SEQ ID NO:1 and (ii) having the following physicochemical properties:

Action: catalyzing the following reaction:

creatine + $H_2O \rightarrow sarcosine + urea$

Heat stability: not more than about 50 °C (pH 7.5, 30 min)

pH stability: being stable at pH 5-8 (40 °C, 18 h preservation)

Km values for creatine in a coupling assay using a sarcosine oxidase and a

peroxidase: 4.5±1.0 mM.

Optimum temperature: about 40-50 °C (at a pH of 6-8 7.5)

Optimum pH: pH about 8.0-9.0 (at a temperature of about 37° C)

Molecular weight: about 43,000 (SDS-PAGE)

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Isoelectric point: about 4.5.

36. (currently amended) A creatine amidinohydrolase (i) encoded by a nucleic acid sequence obtained by mutation of (a) the nucleic acid sequence of SEQ ID NO:2 or (b) a nucleic acid sequence encoding the amino acid sequence of SEQ ID NO:1 and (ii) having the following physicochemical properties:

Action: catalyzing the following reaction:

creatine + $H_2O \rightarrow sarcosine + urea$

Heat stability: not more than about 50 °C (pH 7.5, 30 min)

pH stability: being stable at pH 5-8 (40 °C, 18 h preservation)

Km values for creatine in a coupling assay using a sarcosine oxidase and a

peroxidase: 6.5±1.0 mM.

Optimum temperature: about 40-50 °C (at a pH of about 6-8 7.5) Optimum pH: pH about 8.0-9.0 (at a temperature of about 37° C)

Molecular weight: about 43,000 (SDS-PAGE)

Isoelectric point: about 4.5.

37. (currently amended) A creatine amidinohydrolase (i) encoded by a nucleic acid sequence obtained by mutation of (a) the nucleic acid sequence of SEQ ID NO:2 or (b) a nucleic acid sequence encoding the amino acid sequence of SEQ ID NO:1 and (ii) having the following physicochemical properties:

Action: catalyzing the following reaction:

creatine + $H_2O \rightarrow sarcosine + urea$

Heat stability: not more than about 50 °C (pH 7.5, 30 min)

pH stability: being stable at pH 5-8 (40 °C, 18 h preservation)

Km values for creatine in a coupling assay using a sarcosine oxidase and a

peroxidase: 9.0±1.0 mM.

Optimum temperature: about 40-50 °C (at a pH of 6-8 7.5)

Optimum pH: pH about 8.0-9.0 (at a temperature of about 37° C)

Molecular weight: about 43,000 (SDS-PAGE)

Isoelectric point: about 4.5.

38. (previously added) A method for producing the creatine amidinohydrolase of claim 24, comprising culturing a microorganism producing said creatine amidinohydrolase in a nutrient medium and recovering said creatine amidinohydrolase from the resulting culture.

- 39. (previously added) A reagent for determination of creatine in a sample, comprising the creatine amidinohydrolase of claim 24, a sarcosine oxidase, and a composition for the detection of hydrogen peroxide.
- 40. (previously added) A method for determining creatine in a sample, which comprises measuring an absorbance of a pigment produced by the reaction of the reagent of claim 39 with the sample.
- 41. (previously added) A reagent for determination of creatinine in a sample, comprising a creatinine amidinohydrolase, the creatine amidinohydrolase of claim 24, sarcosine oxidase, and a composition for the detection of hydrogen peroxide.
- 42. (previously added) A method for determining creatinine in a sample, which comprises measuring an absorbance of a pigment produced by the reaction of the reagent of claim 41 with the sample.